THE RATE OF REPRODUCTION OF VARIOUS CONSTITUENTS OF THE BLOOD OF AN IMMUNISED HORSE AFTER A LARGE BLEEDING

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THE RATE OF REPRODUCTION OF VARIOUS CONSTITUENTS OF THE BLOOD OF AN IMMUNISED HORSE AFTER A LARGE BLEEDING.¹

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In physiological literature there are many observations on the effects of bleeding on the various constituents of the blood, and in the literature of immunity there are many publications dealing with the effect of bleeding on the antibody titre in immunised animals. These observations have in large part been made upon goats, rabbits, etc., so that, having the opportunity, it seemed worth while to repeat the observations on horses, from which one can obtain much larger quantities of blood for determinations and so work with a smaller experimental error.

The results of observations on horses possess, moreover, great practical value, as the animals are utilised in the manufacture of sera for therapeutic purposes.

METHODS.

The specific gravity was obtained by weighing 10 c.c. of blood taken from the jugular vein immediately into a gravity bottle kept at room temperature, which varied during the experiments from 45° F. to 56° F.

The duplicate readings are given in the tables. In Figs. 1, 2, 3, 4 the last

two figures are the basis on which percentages were estimated.

Red and white cells.—Quantities of 1 c.c. of blood were diluted and

counted in a modified Bürker apparatus.

The volume of red cells.—After trials with the centrifuge the method adopted consisted in reading the height of the red cell column in a 50 c.c. tall graduated cylinder after standing forty-eight hours at room temperature, by which time the volume had become constant.

Hæmoglobin was estimated by the Haldane-Gowers' apparatus—human

scale.

The above constituents were all estimated in duplicate or triplicate, as will be seen from the table,—the average experimental errors being, for specific gravity, 2 per cent.; red cells, 5 per cent.; white cells, 5 to 10 per cent.; hæmoglobin, 2 per cent.

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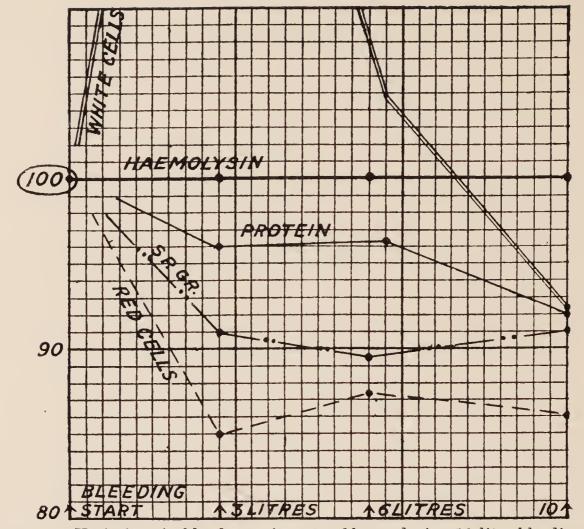


Fig. 1.—Variations in blood constituents of horse during 10-litre bleeding.

Time taken, fifteen minutes. Horse 1.

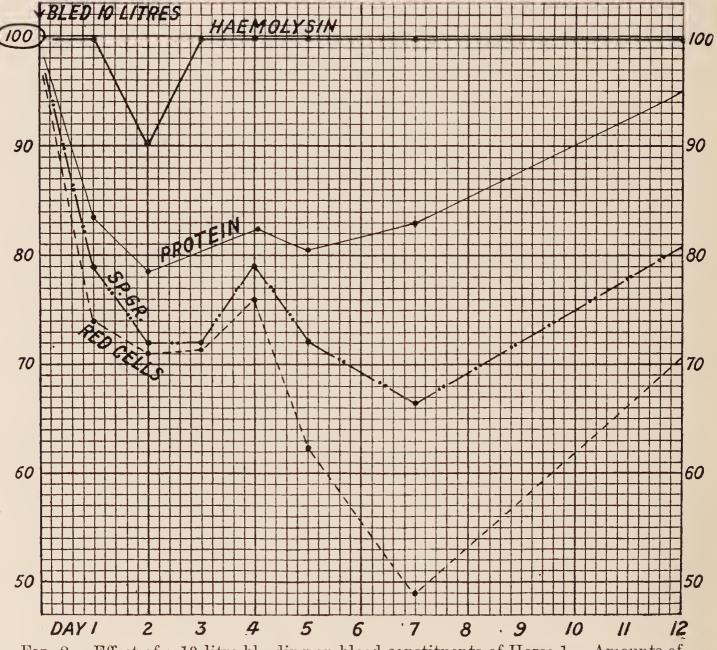


Fig. 2.—Effect of a 10-litre bleeding on blood constituents of Horse 1. Amounts of constituents expressed as percentage of amount present before bleeding.

The total proteins in serum were estimated daily by the refractometer. The refractometer readings were checked at various points by coagulating the

total proteins and weighing the precipitates.

For estimating hæmolytic titre, 0.5 c.c. of 1 per cent. suspension of sheep's red cells containing 26,000 to 28,000 cells per c.c. was employed, 0.05 c.c. fresh guinea-pig serum as complement, and varying dilutions of the horse's serum were added, the whole being made up to 2.5 c.c. with saline solution and incubated for one hour in a water bath at 37° C.

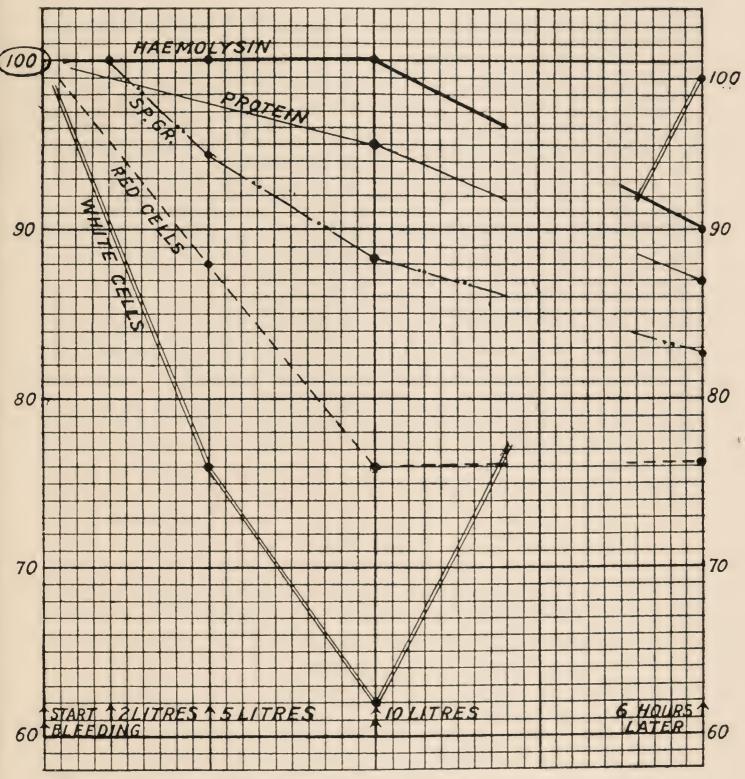


Fig. 3.—Variations of blood constituents of Horse 2 during a 10-litre bleeding, and six hours later.

The samples kept in the cold room were titrated in batches of three, and finally the whole series in one experiment, so that each sample has been

Each tube in the series contained 10 per cent. less horse serum than the preceding. A "standard" old stable hæmolysin was used throughout, so that variations in complement on the various days were provided for, and each day's sample was read as a percentage of the stable hæmolysin.

No wide discrepancies in readings were noted on the various occasions. The quantities given in the tables represent the mean of two or three readings

in each case.

The average error of any point on the curve is from 5 per cent. to 10 per cent. Diphtheria antitoxin was estimated in the usual way, the individual reading being correct to 5 per cent.

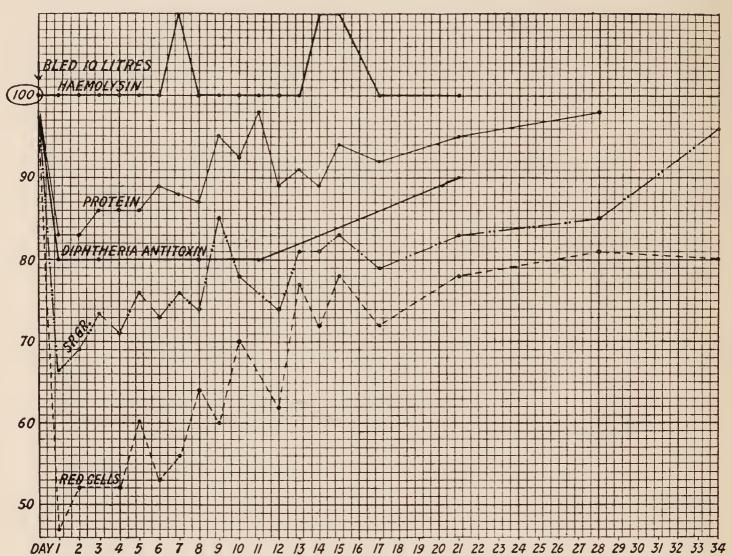


Fig. 4.—Effect of a 10-litre bleeding on blood constituents and antibodies,—hæmolysin and diphtheria antitoxin. Horse 2. Amounts of constituents expressed as percentages of amount present before bleeding.

RESULTS.

The actual results obtained appear in Tables I. and II., and some are expressed graphically in Figs. 1, 2, 3, and 4, for the construction of which the amount of each constituent at the commencement of bleeding is considered to be 100 per cent.

The amount of blood removed (10 litres) was approximately one-fourth of the total blood volume, for although I have not yet succeeded in satisfactorily measuring the total blood volume, estimates obtained by bleeding out and by intravenous injection of known volumes of saline solution justify the assumption that the blood volume of horses of the same weight as those used is from 36 to 40 litres. The weight of Horse 1 was 680 kilos.

It is stated by Athanasiu (1909¹) that the blood volume is the first of the blood constituents to regain the normal figure. If this be so, one would expect, after the removal of 10 litres, i.e., 25 per cent. of the total blood volume, that fluid would pour into the blood vessels until the volume was re-established, at which stage the blood constituents should be about 75 per cent. of the initial figure. Yet in the case of Horse 1 the hæmoglobin and red cells drop on the third day

to about 70 per cent. of the prehamorrhagic level, and in Horse 2 to below 50 per cent. on the second day, the figure for the total proteins being respectively 73 per cent. and 83 per cent. Assuming that the blood volume returns to the initial figure in two days, the 50 per cent. fall in red cells due to the loss of 10 litres of blood in the case of Horse 2 would give by calculation 20 litres for the total blood volume.

Table I.—Results of 10-litre Bleeding. One Year after Immunisation.

Horse 1.

	Specific Gravity of Blood.	Red Cells, Millions per c.mm.	Hæmo- globin.	Volume of Red Cells per cent. of Total Blood.	Leuco- cytes per c.mm.	Total Protein per cent.	Hæmolytic Titre.
At start of bleeding	1057:7	8960 9200 8320 100	104	49 100	6050 6600 100	7·2 100	0·0022 100
After 3 litres	1052.6	7600 7500 85	96 92	43.5 88	9000 8900 142	6·92 96	0.0022 0.002 100
After 6 litres	1051.2	8160 7440 87·5	93 89	4.4 89•5	7000 6250 105	6.95 96.2	0.0022 100
After 10 litres	1052	7520 8080 86	90 86·5	44 89•5	5250 6400 92	6.65 92.5	0·002 0·0022 100
Day 1	1045°8 1045°5 79	6880 6560 74	73 76 71	76 67	7600 \$800 124	5·9 83·3	0.0022 0.002 100
DAY 2	1041·2 1042·1 72	6420 6080 71	64 66 62·5	30 61	\$800 \$100 133	5.65 78	0.0024 90
DAY 3	1043·8 1041·6 72	6400 6240 71.5	76 80 75	35·5 72·5	6500 6500 100	5.8 80 .5	0.0024 0.0022 100
DAY 4	1046 1045 79	6960 6600 76	68 70 70 67·5	33 67	\$700 7800 130	5°94 82°4	••
Day 5	1041.6 1041.6 72	5680 5540 62.5	72 71 68·2	31.8 31.3 64.8	7600 6200 110	5·8 80·5	0:0022 0:002 100
		Hors	SE SUFFERING	FROM MAMMI	TIS.		
DAY 7	1038·4 1038·4 66·5	4240 4400 49	54 52 51	27 55	9300 8800 144	5.98 83	0.0022 0.002 100
Day 12	1047:9 1045:8 80:5	6320 6160 70·5	74 77 72	34:5 71	8600 8500 74	6.85 95	0·0022 100
DAY 15	1055 1053 96·5	8160 8080 92	93 95 90	44 89•5	6000 6200 100	6·85 95	0·0022 100
Day 47	1050 1048 86	8360 8240 95	\$5 \$7 \$7 83.5		3750 4000 61		0.0022

The figures in heavy black represent percentages of the quantity of the particular constituent present at the commencement of the experiment.

Table II.—Effects of 10-litre Bleeding. One Year after Immunisation.

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		Hæmo- lytic	litre.	0.0081 0.0078 0.006 0.008	100 0.0087 0.0097	001	0.0078 0.0098 0.006	0.0078 100	0.0081 100	0.0081 0.013(?) 100	0.0097	100 0·0081 0·013 (?) 100
		, 	Mast Cells.	H	:	•	Н	П	:	:	П	•
		Percentages.	Eosino- phil Cells.	ਨ 4	•	:	ro &	₹. 5.	¢3	c1	1.5	7.0
	Leucocytes.		Mono- nuclear Cells.	1.61	:	:	1-	10	19	55	18	21.4
	Le		Polymorpho- nuclear Cells.	81.5	:	:	% 20	82	62	92	\$0.5 2	92
			Total Counted.	367	:	:	346	353	378	395	372	275
		Diphtheria Anti-toxin Units per	Serum.	50 100	•	:	:	:	40 80	•	40 80	:
	Total Protein in Serum.			6.82 100	:	•	6·52 95	5.94 87	5·65 83	5.7 83	5·94 86	5.9 86
		Leuco- cytes	per c.mm.	9500 9600 100	:	7300	70 5600 5800 62	9200 9600 99	7200 6600 72	6600 7600 74	9300 9500 98	7600 7600 80
		Volume of Red Cells per cent.	or rotal	41.6 100	:	:	42 101	37.2 89	24 58	27 65	27 65	25 60
	Hæmo- globin.		:	104 100	96 95	. 88 8 84 08	72 74 71	50 50 50 8	55 56 50 53	80 80 28	50 49	
	Specific Red Cells, Gravity Millions of Blood. per c.mm.		per Cumi.	9520 9320 100	:	8320 8240	7280 7280 7320 7040	7200 7200 6960	4720 4400 47	4720 5220 52	4800 4772 5400 69	4840 4960 52
			•	1055 100	1052 94·2	1049 1048 88·2	45 46 82.8	36 37 66·3	& & & 6	40 41 73·6	39 38 70.7	
				At start of bleeding .	After 2 litres	After 5 litres	After 10 litres	Six hours later	Day 1 (24 hours later)	Day 2	Day 3	Day 4

0.0097 0.01 0.0081 100	0.0097 0.0097	0.0081 0.0081	0.0097	0.0097	0.0097	0.0097	0.0097	0.0051	0.0021 110	0.0073 0.0081 110	0.0031	0.000	<u>;</u>	:
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•	2.3	63	2,3	က္	÷	က	ç; 61	67.60	7.6	•	•	:	10	
:	17.3	55.3	20.3	20.1	20.3	1.20	6.15	21.8	18	:	:	<u></u>	:	16
:	80	75.2	11	76.3	75.5	4.7	2.92	15	2.62	*		71.5	•	78.5
	375	398	389	393	398	† 0	392	400	37.7	•	:	419	*	393
:	:	÷	40 80	;	:	40 80		•		:	:	45 90	•	:
5·87 86	6.12	6.09	87	6.52 95	6.3 92.5	6.7.5 98	68 80.9	6.25 91	6.12	6.45 94	6.32	6.5 95	86	:
\$300 7900 86	86	7800 8500 85	\$300 7500	9500 9500 83	8500 8500 89	\$600 \$100 88	5800 6200 63 00	9300	\$300 \$700	8300 87	9800	1040 1030 109	7800	1050 9800 106
31	88 88	32 77	88 88	31	69	36 86	91 92 8 75 75 75	5 th th th	29 71	38.5 92	30.5	31.5	36.8	89 36.8 89
58 57	54	9 9 9 7 7 9 9	3 3 8 6	000000000000000000000000000000000000000	69 68 65	882 79	59	1000	9 61 6	57.7. 4	655	4496	2 2 2 3	86 86 86 86 86
5900 5440 5640	5250 4800	5400 5320 566	6400	5680 5720	6400 6950 70	:	4960 5250	0000 0000 01009	5840 6000	6400 78	5840 6000	0400 0480 0480	6480 6950	7360 7840 80
41 42 76	077	<u>4</u> 4 4 5	74.1	4 4 %	3 # # # E	≈ 01 € 4 €	0 + + t	4 4 4 0 4 5 4 4	0 1 4 8 2 4 7 5 1	79998 9998	0 4 4 € 0	त्र भी का व्य प्रमाणिक स्थान	8 4 4 8	55 52 96
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			٠				٠		٠		٠	•	٠	•
DAY 5	Day 6	Day 7	DAY 8	DAY 9	Day 10 .	DAY 11 .	Day 12 .	Day 13	Day 14	DAY 15	DAY 17	DAY 21 .	DAY 28 .	Day 34

The figures in heavy black represent percentages of the quantity of the particular constituent present at the commencement of the experiment.

We know from bleeding out experiments on many horses that the total blood volume is much more than 20 litres, therefore there must be either a hyperhydræmia or a destruction of red cells in the body.

I did not observe hæmoglobinæmia at any time, nor can I find any records by other workers of hæmoglobinæmia or hæmoglobinuria following bleeding, so that we are justified in discarding the hypothesis of destruction of red cells. An actual increase in total blood volume similar to that found by Boycott (1909²) for a week after a large bleeding in a rabbit seems to occur.

On the other hand, the protein content in both horses fell much less than the red cell or hæmoglobin percentage, and less than one would expect it to fall after a 10-litre bleeding if the total blood volume returned to normal in two days. The inference follows that the fluid first sucked from the tissues into the blood stream after a bleeding contains considerably more than the normal amount of protein. In considering these points, it must be remembered that both horses had a history of large bleedings, and may have developed a mechanism differing somewhat from that of normal animals.

Hæmoglobin.—Number and size of red cells.—The percentages representing number of red cells and of hæmoglobin almost coincide throughout, whence it appears that the newly produced red cells contain on the average the normal amount of hæmoglobin. The figures representing volume of red cells do not always agree closely with those of red cells and hæmoglobin. It seems that the average volume of the newly produced red cells may vary somewhat from the prehæmorrhagic level.

The curve of *specific gravity* runs parallel to that of the red cells. This is to be expected, for, of the other two constituents affecting it, the *salts* do not vary much, and the total protein weighs much less than the red cells.

The salts in the case of Horse 1 were estimated cryoscopically, by conductivity, and by direct titration of free chlorides. The amount of total salts does not decrease, but is actually above the prehæmorrhagic figure (as was found by Loeper, 1903³), so that the fluid taken up by the blood after the bleeding contains at least the same amount of total salts as does ordinary serum.

The total protein figures are considered under "Total blood volume."

The white cells content showed considerable irregularities,—one

horse manifesting great leucocytosis during bleeding, the other considerable leucopenia, the curves of both horses showing the widest irregularities during the recovery period. The samples could not be taken at the same hour every day, the variations being between 11 a.m. and 5 p.m., but the wide fluctuations on the curves do not show any definite relationship to the times at which the samples were taken.

The differential counts show remarkably little variation, the discrepancies being very little outside the experimental error.

Antibodies.—A comparison of the curves of total protein, hæmolysin, and diphtheria antitoxin furnishes the most interesting of the results. Of the various titrations of hæmolysin there is scarcely a reading more than 10 per cent. (i.e., one test tube in the series) on either side of the figure representing the hæmolytic titre before the bleeding. After making all allowances for experimental error, it is certain that the curve of hæmolytic titre does not run parallel to that of total protein, and that the titre remains almost constant throughout, not-withstanding fluctuations in total blood volume. This result holds good in both horses, and is therefore probably more than a coincidence.

The curve of diphtheria antitoxin titre, on the contrary, runs nearer to that of the total protein curve throughout in Horse 2. The obvious inference is that the protein first reproduced after the bleeding contains more hæmolysin than existed in the serum protein before the bleeding, and more than in that produced later, but a proportion of diphtheria antitoxin not widely different from that in the serum protein present before the bleeding, and that the various "protein fractions" containing different amounts of various antibodies are produced at different rates. The bearing of this latter suggestion is to be the subject of a further research.

SUMMARY OF RESULTS.

After taking 10 litres of blood from an immunised horse the following results occur:—

- 1. The number of white cells varies widely and irregularly. Very small differences are found in the differential count.
- 2. The hæmolytic titre remains practically constant, showing variations of at most 10 per cent. from the initial figure.
- 3. The total amount of salts present does not decrease, but may be increased 10 per cent.
- 4. The content of all other blood constituents falls, the hæmoglobin and number of red cells falling together to 50 per cent. or 60 per cent. of the initial figure. The colour index of the new red cells remains at the normal figure. The curve of volume of the red cells does not correspond very closely with that of the number of red cells.
- 5. The curves of total proteins and of diphtheria antitoxin show a fairly close relationship.

Conclusions.

After the withdrawal of 10 litres of blood from an immunised horse, the fluid first appearing in the blood vascular system contains an amount of protein far above the normal, hæmolysin and diphtheria

antitoxin are reproduced at different rates, and the rate of reproduction of the various proteins in the blood is probably associated therewith.

I have to thank Dr. Walpole for the whole of the cryoscopic, conductivity and protein determinations.

REFERENCES.

1.	Athanasiu.		•	"Dictionnaire de Physiologie,"	1909, tome
2.	BOYCOTT AND	Douglas .	•	viii. p. 514. Journ. Path. and Bacteriol.,	Cambridge,
				1909, vol. xiii. p. 271.	0,
3.	Loeper			Cited by Athanasiu, p. 490 (vide a	supra).



